

GENETIC ENGINEERING AS AN ALTERNATIVE TO METHYL BROMIDE FUMIGATION

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Plant genetic engineering is the modification of a plant through the introduction of novel genes. Plant genetic engineering can be used for the development of alternatives to methyl bromide fumigation in two scenarios. In one, a genetically engineered (transgenic) plant would contain genes that confer resistance to fungal pathogens, nematodes and herbicides, so that no fumigant would be needed. Secondly, transgenic plants could be used in combination with existing alternative fumigants, such as a herbicide resistant plant in combination with Telone.

The first step of the genetic engineering, the identification and isolation of genes that confer the necessary resistance traits, is currently the most difficult step toward development of methyl bromide alternatives. Once isolated, these genes can be modified and introduced into plants using a suite of relatively standard molecular tools, which include:

1. Manipulation of the structure of the gene
2. Introduction of the modified gene into the target plant
3. Analysis of the transgenic plant and identification of those with desired properties

Each of these steps will be described in detail, followed by an update on the development of the molecular tools for strawberry, and progress towards engineering each of the three traits.

1. Gene modification

The isolated gene is modified through the addition of controlling elements i.e. promoters and terminator sequences, in order to customize the expression of the gene. A number of promoters are available to target expression to specific tissues and/or to provide a high level of gene expression. For example, high expression in the roots would be used for nematode resistance and root-rot fungal pathogen resistance, while over-the-top application of herbicides requires expression of the resistance gene throughout the plant.

2. Gene introduction

The resulting transgenes are introduced into plants mainly through ballistic or *Agrobacterium* transformation. *Agrobacterium tumefaciens* is the causal agent of Crown Gall. During infection in the field, a portion of *Agrobacterium* DNA is introduced into the genome of the host plant. This DNA, the T-DNA, is a very precisely defined section of DNA bordered by two segments that flank the genes responsible for Crown Gall. Using disarmed *A.tumefaciens* strains in which the disease genes have been removed, the transgene is inserted between the T-DNA borders and used to infect plant tissue. During incubation, the *Agrobacterium* introduces the T-DNA containing the transgene into the plant genome. In order to select the relatively few cells that have been infected and carry the new gene, a selectable marker gene, e.g. the genes conferring kanamycin or chlorsulfuron resistance (NPTII or surB respectively) are attached to the gene of interest.

On media containing the selective agent, only those cells containing the transgenes will survive. Once transformed cells are obtained, plants are regenerated from those cells by manipulating hormone concentrations in the growth media.

3. Plant analysis

Plants are analyzed for presence and correct integration of the T-DNA, optimum expression of the transgene, absence of bacterial sequences from beyond the T-DNA borders, lack of somaclonal variation and for desired phenotype.

Strawberry Genetic Engineering Tools.

All necessary genetic engineering tools are available for strawberry. Strawberry is very amenable to *Agrobacterium* transformation and efficiencies are high. Plant regeneration is very efficient from both greenhouse and *in vitro* grown leaf material. Many diverse cultivars, both short day and day neutral, have been transformed. Promoters that provide expression in fruit tissue and promoters that provide high level expression throughout the plant are available. Molecular methods to characterize transgenic plant sequences and gene expression have been adapted to strawberry tissue.

Genes and Traits.

1. Weed control.

In strawberry, the loss of the herbicidal component of methyl bromide will have greatest impact in the nurseries, as cultural weed control practices are not possible. Without some form of weed control, certification and clonal integrity could be compromised, and yields would be reduced due to competition and increased viral diseases. Weed control through genetic engineering has been achieved in a number of crops through the introduction of the genes for glyphosate resistance. These Roundup Ready crops e.g. soybeans, cotton, corn, and canola, can resist over-the-top application of Roundup herbicide. Roundup Ready strawberries combined with, for example Telone C17, would provide an excellent alternative to methyl bromide.

2. Fungal Disease Resistance

For effective fungal disease control, a strategy combining genes conferring broad-spectrum resistance would be necessary. Transgenic strawberries would need to be tolerant to *Verticillium*, *Phytophthora* sp., *Pythium* and others. A number of different genes have been introduced into plants and shown to provide resistance to one or more fungi. For example tobacco and tomato plants transformed with a stilbene synthase gene from grape synthesize the antifungal compound resveratrol, and show increased tolerance to *Botrytis* and *Phytophthora*. *Arabidopsis thaliana* transformed with a controlling gene for systemic acquired resistance, *npr1*, shows increased tolerance to a range of pathogens including *Peronospora*. These genes and others differ in their mode of action, and introduced together, have the potential to give broad fungal and perhaps bacterial resistance.

3. Nematode Resistance

Strawberry is affected primarily by the nematode *Meloidogyne hapla*, though the other *Meloidogyne* species are found. Nematode control would need to be broad to provide tolerance to the range of nematodes present in soil. A number of strategies are being investigated in the search for transgenic nematode control e.g. isolation of resistance

genes from wild relatives of crops of interest e.g. Mi and Hero from tomato, cre3 from wheat; isolation of nematode controlling Bt genes etc. The best results have been obtained in *Arabidopsis* in which transgenes producing protease inhibitors give increased tolerance to a range of nematodes. Also promising is the use of antisense aquaporin genes in tobacco and cotton to give enhanced tolerance to rootknot nematode. Some of these genes, or their strawberry equivalent, may be effective for nematode control in strawberry.

Of the three traits required for methyl bromide replacement, herbicide resistance through the production of a Roundup Ready strawberry would probably be the first to commercialization. The strategy has been proven in other crops and the engineering tools are in place. Prospects are good for fungal resistance, as a number of genes have shown efficacy against a range of fungi. The introduction of multiple genes simultaneously in order to obtain broad-spectrum resistance is still being tested. Early results with some nematode resistance strategies are encouraging, though obtention of this trait is likely to be the most long-term.